2008

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Publication Info

Postprint version. Published in *Journal of Nutrigenetics and Nutrigenomics*, Volume 1, Issue 4, 2008, pages 196-204.
Steck, S. E., Keku, T., Butler, L. M., Galanko, J., Massa, B., Millikan, R. C., & Sandler, R. S. (2008). Polymorphisms in methionine synthase, methionine synthase reductase and serine hydroxymethyltransferase, folate and alcohol intake, and colon cancer risk. *Journal of Nutrigenetics and Nutrigenomics, 1*(4), 196-204.
DOI: 10.1159/000136651

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Published in final edited form as:
J Nutrigenet Nutrigenomics. 2008 ; 1(4): 196–204. doi:10.1159/000136651.

Polymorphisms in Methionine Synthase, Methionine Synthase Reductase and Serine Hydroxymethyltransferase, Folate and Alcohol Intake, and Colon Cancer Risk

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Abstract

Background/Aims—We examined associations among folate and alcohol intake, SNPs in genes involved in one-carbon metabolism and colon cancer risk.

Methods—Colon cancer cases (294 African Americans and 349 whites) were frequency matched to population controls (437 African Americans and 611 whites) by age, race and sex from 33 North Carolina counties from 1996 to 2000. Folate and alcohol intakes were collected by dietary interview. Five SNPs were genotyped using DNA from whole blood: *SHMT C1420T; MTRR A66G; MTR A2756G*, and the previously-reported *MTHFR C677T* and *MTHFR A1298C*. Adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression.

Results—An inverse association was observed for *SHMT TT* genotype as compared to *CC* genotype in whites (OR=0.6, 95%CI=0.4, 1.0), but not in African Americans. Inverse associations were observed for high folate intake in individuals carrying 0 or 1 variant allele [OR 0.2 (95%CI 0.06 – 0.8) for African Americans; OR 0.2 (95%CI 0.1– 0.6) for whites] compared to low folate intake. Modest interactions between these SNPs and alcohol or folate intakes were observed.

Conclusions—Our results are consistent with other findings and provide needed data on these associations among African Americans.

Keywords

Alcohol; case-control study; colon cancer; folate; polymorphism

Introduction

Low folate and high alcohol intakes are associated with an increased risk of colon cancer [1]. Folate is critical to one-carbon metabolism, acting as a coenzyme in nucleotide synthesis and DNA methylation. Folate levels may be depleted with high alcohol intake [2]. While folate has

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generally been thought to be safe and protective against cancer, there is evidence that it can promote the growth of preneoplastic lesions and recent work has emphasized the importance of timing and dose of folate in the carcinogenesis process [3]. In fact, investigators of a double-blind, randomized, placebo-controlled clinical trial of recurrent colorectal adenomas recently reported that folic acid at 1 mg/d for six to eight years did not reduce risk of recurrent colorectal adenoma and tended to increase the risk of advanced lesions and adenoma multiplicity [4]. Thus, investigating the mechanism of action of folate and enzymes involved in folate metabolism is important for enhancing our understanding and improving the effectiveness of our public health recommendations.

The folate metabolism pathway is complex and involves various enzymes that regulate DNA synthesis and DNA methylation. Among others, 5,10-methylenetetrahydrofolate reductase (MTHFR) is required for methylation of homocysteine to methionine [5]; serine hydroxymethyltransferase (SHMT) catalyzes the reversible conversion of serine and tetrahydrofolate to glycine and methylene tetrahydrofolate; methionine synthase (MTR) supports methionine homeostasis by catalyzing the remethylation of homocysteine to methionine in a cobalamine-dependent reaction that utilizes MTHFR as methyl donor [6]; and methionine synthase reductase (MTRR) is responsible for keeping MTR in an active form by maintaining adequate levels of activated cobalamin, the enzyme cofactor for MTR. Single nucleotide polymorphisms (SNPs) in the genes that encode these enzymes may modify the effect of folate or alcohol intake on colon cancer [7].

We previously reported associations among two polymorphisms in the *MTHFR* gene, folate and alcohol intake, and colon cancer risk in the North Carolina Colon Cancer Study (NCCCS) [8]. We genotyped SNPs in three additional genes encoding enzymes involved in folate metabolism, *MTR*, *MTRR* and *SHMT* to examine individual gene effects and joint effects with folate and alcohol intake on colon cancer risk in an ethnically diverse population. We chose these genes because they are important in folate metabolism. Our *a priori* hypothesis was that diet and genes would act synergistically such that individuals with the combination of high folate intake or no alcohol intake and increased number of variant alleles as defined by previous functional and epidemiologic literature would be at the lowest risk for colon cancer as compared to individuals with low folate intake or ever alcohol intake and fewer number of variant alleles.

Materials and Methods

The NCCCS has been described in detail previously [8]. Briefly, a case-control study of colon cancer was conducted in 33 counties in North Carolina between July 1, 1996 and June 30, 2000. Individuals with a first diagnosis of histologically confirmed invasive adenocarcinoma of the colon between July 1, 1996 and June 30, 2000 were identified through the rapid ascertainment system of the North Carolina Central Cancer Registry. Other case eligibility criteria included age between 40 and 85 at the time of diagnosis, residing in the 33-county study area in North Carolina, ability to give informed consent and be mentally competent to complete the interview, have a North Carolina driver's license or identification card if <age 65 (because controls <age 65 were sampled from driver's license rosters), and permission from their primary physician to participate in the study. Controls were frequency matched to cases by race, sex, and by 5-year age group by sampling from two sources: 10 people younger than age 65 from Division of Motor Vehicle record of individuals with North Carolina driver's license or state identification cards, and 2) people age 65 and older from a list of Medicare eligible beneficiaries obtained from the Health Care Financing Administration. As described previously, for cases the contact rate (contacted participants/eligible) was 78%, the cooperation rate (interviewed/eligible) was 84% and the overall response rate (product of the contact and cooperation rates) was 66%. For controls, the contact rate was 90%, the cooperation rate was 62% and the overall response rate was 56% [8]. 643 colon cancer cases (294 African Americans

and 349 whites) and 1048 population-based controls (437 African Americans and 611 whites) were enrolled in the study.

Alcohol and folate (both dietary and supplemental) intakes were assessed using an interviewer-administered Block food frequency questionnaire that was modified slightly to include regional and ethnic foods commonly consumed in a Southern population [9]. Dietary folate was estimated from the folate content, amount, and frequency of consumption for folate-containing foods on the FFQ. Participants were asked if they took supplemental folate or multivitamins on a regular basis. Individual supplemental folate intake was estimated from the formulation of the most common over-the-counter multivitamin supplements used in the study population (400ug folate). Only 31 participants, or 2% cases and 2% controls, reported taking folate supplements alone (i.e., as a single vitamin, not in a multivitamin). In summary, total folate intake was defined as the sum of dietary and supplemental folate intake, as recommended by the Food and Drug Administration [10].

Blood samples were obtained from 86% of cases and 83% of controls from which DNA was extracted for genotyping. Cases and controls who provided blood samples were more likely to be male, white, and never-smokers ($p<0.01$), as previously reported [8]. No other differences between those who provided blood samples and those who did not were noted for variables such as age, education level, income, family history of colorectal cancer, or total meat intake. Five SNPs in four genes involved in the folate metabolism pathway were genotyped. The genotyping assays for the *MTHFR C677T* (rs1801133) and *A1298C* (rs1801131) polymorphisms have been described previously [11].[5] For the genotyping of *MTRR A66G (rs1801394)*, *MTR A2756G (rs1805087)* and *SHMT C1420T (rs1979277)*, TaqMan® probes and primers were based on the genetic sequences from the SNP500 website (www.SNP500cancer.nci.hin.gov) and the TaqMan® assays were designed using the Applied Biosystems' Assays-by-Design^{SM} service. PCR reactions consisted of unlabeled PCR primers, allele specific TaqMan® MGB probes (FAM™ and VIC® dye-labeled), 1x TaqMan® Universal PCR Master Mix, No AmpErase® UNG, and 15 ng of genomic DNA. PCR reactions were performed in a 15.0 uL reaction volume using the Hot-Start format. PCR amplification was run on a Perkin Elmer GenAmp® 9700 thermocycler using the 9600 mode under the following conditions: 1 cycle of 50.0° C for 2 minutes (AmpErase® UNG Activation), 1 cycle of 95.0° C for 10 minutes (AmpliTaq Gold Activation), followed by 40 cycles of 92.0° C for 15 seconds of denaturation and appropriate annealing temperature for 1 minute.

Genomic controls obtained from the Coriell Tissue Repository (Camden, New Jersey) were used as positive controls. Samples that did not amplify or could not be scored were repeated. Samples that did not amplify on the second run were scored as undetermined. For quality control, a randomly selected 10% of samples were repeated. All of the 10% repeat results were found to be identical to the initial analysis. Hardy Weinberg Equilibrium was examined for each SNP using a goodness of fit χ^2 test to compare the observed genotype frequencies with expected genotype frequencies based on the observed allele frequencies.

Differences between cases and controls with regard to descriptive variables and risk factors for colon cancer were compared using a t-test for continuous variables and a chi-square test for categorical variables. Percentages or means ± standard errors are presented. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) using SAS statistical software version 8.0. Models presented here are adjusted for offset terms (to account for randomized recruitment scheme and to maintain the efficiency benefits of matching) related to the age, race and gender sampling probabilities [12] used to identify eligible participants. Folate intake was categorized into "low" versus "high" intake using the current US Dietary Reference Intake value (low is defined as less than 400ug, high is defined as equal to or greater than 400ug) as the cut-off. We also examined folate intake categorized

as a tertile variable, using the 33%-ile and 67%-ile cutpoints among control subjects only. Joint effects with genotype did not differ using the three-level folate intake variable as compared to the dichotomous variable, so only the dichotomous variable results are presented in this paper. Due to the low reported consumption of alcohol in this population, alcohol intake was defined as "drinker" or "non-drinker."

We evaluated whether the joint effects represented departures from the additive and/or multiplicative model, because it has been suggested that joint effects that are less than multiplicative, but greater than additive may still be relevant, especially in terms of public health significance [13;14]. Joint effects of genotype and folate or alcohol intake were examined by using low folate or drinker, respectively, and the genotypes' hypothesized highest risk category (based on previous experimental and epidemiologic literature) as the common referent group and calculating ORs for each combination of folate or alcohol intake and genotype. Interaction contrast ratios (ICRs) and 95% confidence intervals were determined to examine additive interaction using the two-level genotype variables (homozygote wildtype vs. heterozygotes+homozygote variant) and folate or alcohol intake. ICR>0 is interpreted as greater than additive joint effects, ICR<0 is interpreted as less than additive joint effects, and ICR=0.0 indicates no departure from additivity. Likelihood ratio tests (LRT) were used to determine fit of the interaction term in the model to assess multiplicative interaction.

A summary SNP variable was created summing the number of variant alleles present for each SNP for each participant. In creating the summary SNP variable, we attempted to group the alleles for each SNP according to *a priori* hypotheses about their functional effects and subsequent effect on cancer risk. For each gene, the variant allele was defined as the less common allele, except for *MTRR 66* where previous literature suggests that the more common allele is associated with reduced risk of colon cancer, in contrast to the other gene SNPs where the more common allele has been associated with increased risk of colon cancer. Thus, for the summary variable, the following definitions of variant allele applied based on functional and epidemiologic data in the literature: *MTHFR 677:T; MTHFR 1298:C; SHMT 1420:T; MTRR 66:A*; and *MTR 2756:G*.

Results

There were 546 cases (44% African Americans) and 855 controls (38% African Americans) with complete genotyping data. Cases were slightly younger than controls (mean age cases 63.8 ± 0.4 versus 65.9 ± 0.3 yrs in controls, p=0.0001), consumed more calories from alcohol (p= 0.0009; mean intake for cases was 64.2 ± 8.0 kcals/d and for controls was 35.8 ± 3.0 kcals/d), used NSAIDs less frequently (p=0.04; mean was 12.6 ± 1.2 NSAIDS used per month for cases and 15.7 ± 0.9 NSAIDS used per month for controls) and tended to consume less folate on average than controls (p=0.07; cases = 397+10 ug/day versus controls = 421+8 ug/day). The test for Hardy Weinberg Equilibrium was satisfied for all SNPs among both case and control subjects separately (p>0.10).

As shown in Table 1, white carriers of the *SHMT 1420TT* genotype had an OR=0.6 (95% CI=0.4, 1.0) as compared to *CC* carriers, while no association was observed in African American carriers of the *TT* genotype (OR=1.1, 95%CI= 0.6, 1.8 as compared to *CC* carriers). No other substantial associations were observed for individual or combined genotypes and colon cancer risk.

Table 2 shows the joint effects of diet and genotype on colon cancer risk by race. For whites, high folate intake was associated with reduced risk of colon cancer among all genotypes for the individual SNPs. ICRs revealed weak departure from additivity for folate and genotypes, the only statistically significant finding was for *MTRR* in whites (p=0.01 for ICR; p=0.04 for

LRT evaluating multiplicative interaction). In both African Americans and whites, the inverse association between high folate intake and colon cancer risk was strongest in those individuals with the least number of variant alleles (OR=0.2 and 95% CIs excluded 1.0 for both ethnic groups comparing high folate intake to low-folate intake with 0 or 1 variant alleles), and LRTs revealed borderline statistically significant departure from interaction on the multiplicative scale (p=0.05 for African Americans, p=0.04 for whites). When dietary folate alone or folate supplement use alone was examined for interaction with genotypes, conclusions did not differ from those presented in Table 2 with the exception that the LRT p-value was no longer significant for the *MTRR A66G* genotype among whites (p=0.10 for dietary folate only and p=0.43 for supplemental folate only).

For African Americans, reduced risk of colon cancer was observed for never drinkers with the *SHMT CC* genotype, and for ever drinkers with the *SHMT TT* genotype, although neither of these associations was statistically significant (Table 3). The ICR>0.5 suggested greater than additive effects (p=0.002 for ICR) and the LRT suggested greater than multiplicative interaction (p = 0.04). There was no evidence for interaction among whites. Due to small numbers, many of the estimates were imprecise when stratified by race.

Discussion

We examined the associations among colon cancer and SNPs in genes encoding three enzymes involved in the folate metabolism pathway, *SHMT*, *MTRR* and *MTR*, based on functional changes of these SNPs and previous reports of associations with colon cancer. With the exception of a borderline statistically significant decreased risk of colon cancer in whites with the *SHMT TT* genotype as compared to the *CC* genotype, we found no association between the individual SNPs studied and colon cancer risk. In combined gene analyses, which also included two previously reported SNPs in the *MTHFR* gene, the inverse association between folate intake and colon cancer was strongest among those individuals with the least number of variant alleles for both African Americans and whites which was contrary to our *a priori* hypothesis of greatest reduced risk among those with the most number of variant alleles and high folate intake.

Genetic polymorphisms in genes that encode key enzymes in the folate pathway, such as *MTHFR*, *SHMT*, *MTRR* and *MTR*, may contribute to colon cancer risk. Functional polymorphisms in these genes may alter the availability of folate for DNA synthesis and methylation, and consequently influence susceptibility to cancer. Functional changes associated with SNPs in these folate metabolizing genes have been reported [7], though the data are limited for most. In one study, *SHMT 1420* CC genotype carriers were found to have reduced plasma folate, reduced red cell folate and increased plasma homocysteine compared to TT carriers [15], though no such associations were found in another study [16]. For the *MTR 2756* A>G polymorphism, decreased homocysteine levels have been found for the GG genotype as compared to the AA genotype in some studies [17–19], but not all [20–22]. For *MTRR A66G*, some studies have reported elevated homocysteine levels for carriers of the homozygote wildtype genotype (AA) as compared to other genotypes [23;24], while others have not [25]. More research is needed regarding the functional effects of SNPs in these genes as related to effects on folate metabolism and subsequent folate availability.

Other epidemiologic studies have found associations between some of these individual SNPs and colon cancer risk, though few studies have examined these in African Americans. The *MTHFR 677 TT* and the *1298 CC* genotypes individually are associated with reduced risk of colon cancer in the majority of studies to date [[26] and reviewed in [7]]. Lowest risk has been found among those with both *MTHFR 677TT* and *1298CC* genotypes [8]. For *MTRR A66G*, an increased risk of colorectal cancer was observed among Japanese with the *GG* genotype

compared to *AA+AG* referent [27], and another study found increased risk with the *GG* genotype among white subjects only [28]. Results of studies of the *MTR 2756 G* allele and colon cancer or colorectal adenoma have been inconsistent [7;27–29], and recent analyses of the Nurses Health Study and Health Professionals Follow-up Study found a nonsignificant increased risk of colorectal cancer for variant allele carriers of this SNP [30].

We observed a modest inverse association for carriers of the homozygous *SHMT C1420T* variant genotype among whites. In two prospective cohort studies of colorectal cancer, no association was reported with the *SHMT* genotype [16]. The difference between our finding and the previous finding could reflect physiological differences in the effects of folate metabolism in the colon versus the rectum. For example, an inverse association between the *MTHFR C677T* genotype has also been reported for colon, but not rectal cancer [31].

We did not observe an association among African Americans for *SHMT C1420T* genotype and colon cancer. Consistent with this finding, we previously reported an inverse association for the *MTHFR 1298 CC* genotype and colon cancer among whites, but no association among African Americans [8]. Differences in folate metabolism between African Americans and whites, other than those due to variation in the measured genes, may explain lack of an association between *SHMT* and colon cancer among African Americans in our study. This hypothesis is supported by findings from a nutritional study in a controlled environment, where African American women had statistically significant lower folate blood and urine levels, compared to non-Hispanic white and Hispanic women, regardless of *MTHFR* genotype or folate intake [32].

There is evidence that associations between some folate-metabolizing enzyme genes and colon cancer are observed only in the context of folate or alcohol intake [33]. We were able to examine joint effects of folate or alcohol intake and genotype on colon cancer risk by race, but were limited by small cell sizes when examining the interaction between these gene-diet exposures. For example, our finding of an inverse association among African American drinkers with the *SHMT TT* genotype was based on only 4 cases, and therefore highly imprecise and likely to be spurious.

A limitation of our study was the opportunity for misclassification of dietary exposures. It is possible that nondifferential recall of folate-containing foods biased our joint effects odds ratios toward the null. Our study design limited the opportunity for misclassification of dietary exposures by using a modified version of an 150-item, validated food-frequency questionnaire that was administered in-person by trained interviewers. Slight modifications to the previously-validated FFQ were made to include foods consumed by a Southern population [9] and these additions were not validated. However, these modifications most likely had minimal or no effect on assessment of folate or alcohol intakes. Vitamin supplement use was evaluated as a source of folate intake, and we estimated the amount of folate intake from individual folate supplements from the formulation of the most common over-the-counter multivitamin supplements used in the study population (400ug folate). Given the small number of participants who reported consuming individual folate supplements (2% of cases and controls), this is unlikely to have biased our results substantially. In addition, we examined the gene-folate interactions using only dietary sources of folate intake and in separate analyses, using only folate supplement use, and our results did not change substantially from those presented here.

Because many genes are involved in folate metabolism, the effect of multiple functional polymorphisms in genes encoding for enzymes in the pathway may be expected to be stronger than the effect of any one individual polymorphism. However, we found no strong evidence for this in our combined analyses by summing number of variant alleles and comparing the

highest category of variant alleles to the lowest. Other studies have examined different combinations of folate-metabolizing enzyme genes including the methylenetetrahydrofolate dehydrogenase (*MTHFDI*), glutamate carboxypeptidase II (*GCPII*), thymidylate syntase (*TS*), and reduced folate carrier (*RFC*) genes [16;34;35] which were not studied here, but no clear patterns have emerged. Koushik et al. reported associations for 24 SNPs in thirteen genes involved in one-carbon metabolism and colorectal cancer risk, and found associations for *MTHFR C677T*, *MTRR Ser284Thr*, *MTRR Arg415Cys* as well as joint effects for *MTR Asp919Gly* and *TCN II Pro259Arg* [30]. Large populations are required to examine these gene-gene and gene-nutrient interactions using traditional epidemiologic methods. New methods of mathematical modeling developed for this pathway may provide insight into the effects of modifying components of the system to inform future studies [36]. Additional studies are needed to replicate our findings in different racial/ethnic groups and improve our ability to predict the effects of polymorphisms within genes in one-carbon metabolism and folate status on colon cancer risk.

Acknowledgments

This research was supported in part by grants from the National Institutes of Health (P30 DK34987, R01 66635, and 1K07CA102640). Dr. Butler was supported by National Institute of Child Health and Human Development's Building Interdisciplinary Research Careers in Women's Health (BIRCWH) grant 5K12HD051958. These analyses were conducted while Dr. Steck was an American Institute for Cancer Research/World Cancer Research Fund Marilyn Gentry Fellow at the University of North Carolina at Chapel Hill.

Abbreviations

CI
: confidence interval

MTR
: methionine synthase

MTHFR
: 5,10-methylenetetrahydrofolate reductase

MTRR
: methionine synthase reductase

NCCCS
: North Carolina Colon Cancer Study

OR
: odds ratio

SHMT
: serine hydroxymethyltransferase

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Table 1

Adjusted ORs and 95%CIs for colon cancer by genotype and race

	African Americans			Whites		
	Cases/Controls	**OR***	**95% CI**	**Cases/Controls**	**OR***	**95%CI**
SHMT 1420						
CC	106/153	1.0	Ref	151/257	1.0	Ref
CT	105/130	1.2	0.9, 1.8	128/205	1.0	0.8, 1.4
TT	28/39	1.1	0.6, 1.8	28/71	0.6	0.4, 1.0
CT, TT	133/169	1.2	0.8, 1.7	156/276	0.9	0.7, 1.3
MTRR 66						
GG	24/26	1.0	Ref	99/168	1.0	Ref
AG	99/127	1.0	0.5, 1.8	155/256	1.0	0.8, 1.4
AA	116/169	0.9	0.5, 1.6	53/109	0.8	0.5, 1.2
AG, AA	215/296	0.9	0.5, 1.7	208/365	1.0	0.7, 1.3
MTR 2756						
AA	129/182	1.0	Ref	205/347	1.0	Ref
AG	94/125	1.1	0.8, 1.5	91/171	0.9	0.7, 1.3
GG	16/15	1.4	0.6, 2.9	11/15	1.2	0.6, 2.8
AG, GG	110/140	1.1	0.8, 1.6	102/186	0.9	0.7, 1.3
# Variant Alleles§						
0 or 1	19/34	1.0	Ref	36/57	1.0	Ref
2 or 3	124/167	1.6	0.9, 3.0	172/255	1.1	0.7, 1.7
4 or 5	87/108	1.7	0.9, 3.3	84/195	0.7	0.4, 1.1
6 or more	9/13	1.5	0.5, 4.4	15/26	0.9	0.4, 1.9

*Adjusted for offset terms, age, and gender

§Includes SNPs listed in table and *MTHFR C677T* and *A1298C*; variant allele defined as follows: *MTHFR 677:T; MTHFR 1298:C; SHMT 1420:T; MTRR 66:A*; and *MTR 2756:G*.

Table 2

Joint effects of individual and combined SNPs and folate intake on colon cancer risk by race

	African Americans						Whites					
	Cases/ Controls	**OR***	**95%CI**	**Cases/ Controls**	**OR***	**95%CI**	**Cases/ Controls**	**OR***	**95%CI**	**Cases/ Controls**	**OR***	**95%CI**
	Low folate <400ug			**High folate >=400ug**			**Low folate <400ug**			**High folate >=400ug**		
SHMT 1420												
CC	72/93	1.0	Ref	31/54	0.7	0.4, 1.2	94/121	1.0	Ref	53/129	0.5	0.3, 0.8
CT	67/85	1.0	0.7, 1.7	34/42	1.0	0.6, 1.8	68/97	0.9	0.6, 1.3	57/100	0.7	0.5, 1.1
TT	19/27	0.9	0.4, 1.7	8/12	0.9	0.3, 2.3	13/32	0.5	0.3, 1.1	15/37	0.5	0.2, 0.9
CT, TT	86/112	1.0	0.7, 1.5	42/54	1.0	0.6, 1.7	81/129	0.8	0.5, 1.2	72/137	0.7	0.4, 1.0
	ICR= 0.34 (95% CI −0.29, 0.96), p = 0.29						ICR= 0.33 (95% CI −0.05, 0.71), p= 0.09					
	LRT p= 0.29						LRT p= 0.13					
MTRR 66												
GG	16/19	1.0	Ref	7/7	1.0	0.3, 3.8	63/74	1.0	Ref	34/91	0.4	0.2, 0.7
AG	67/78	1.2	0.5, 2.5	27/44	0.8	0.3, 1.8	82/126	0.8	0.5, 1.2	69/121	0.7	0.4, 1.0
AA	75/108	1.0	0.4, 2.0	39/57	0.9	0.4, 2.1	30/50	0.7	0.4, 1.2	22/54	0.5	0.3, 0.9
AG, AA	142/186	1.0	0.5, 2.2	66/101	0.9	0.4, 1.8	112/176	0.7	0.4, 1.1	91/175	0.6	0.4, 0.9
	ICR= −0.24 (95% CI −1.71, 1.23), p= 0.75						ICR= 0.44 (95% CI 0.09, 0.78), p= 0.01					
	LRT p= 0.71						LRT p= 0.04					
MTR 2756												
AA	87/114	1.0	Ref	36/62	0.7	0.4, 1.2	121/164	1.0	Ref	82/168	0.6	0.5, 0.9
AG	64/81	1.0	0.7, 1.6	28/41	0.9	0.5, 1.6	48/80	0.8	0.5, 1.3	39/89	0.6	0.4, 0.9
GG	7/10	0.8	0.3, 2.3	9/5	2.0	0.6, 6.4	6/6	1.3	0.4, 4.3	4/9	0.6	0.2, 2.1
AG, GG	71/91	1.0	0.7, 1.5	37/46	0.7	0.6, 1.7	54/86	0.9	0.6, 1.3	43/98	0.6	0.4, 0.9
	ICR= 0.29 (95% CI −0.36, 0.95), p= 0.38						ICR= 0.07 (95% CI −0.39, 0.53), p= 0.77					
	LRT p= 0.36						LRT p= 0.89					
# Variant Alleles[§]												
0 or 1	15/18	1.0	Ref	4/15	0.2	0.1, 0.8	26/24	1.0	Ref	9/31	0.2	0.1, 0.6
2 or 3	81/107	1.0	0.4, 2.1	36/55	0.8	0.4, 1.9	99/114	0.8	0.4, 1.4	69/134	0.5	0.2, 0.8
4 or 5	58/70	1.1	0.5, 2.3	28/36	1.0	0.4, 2.3	43/98	0.4	0.2, 0.8	39/89	0.4	0.2, 0.8
6 or more	4/10	0.5	0.1, 2.0	5/2	3.4[£]	0.6, 20.3	7/14	0.5	0.2, 1.3	8/12	0.5	0.2, 1.6

	African Americans				Whites			
	Cases/ Controls	OR*	95%CI	Cases/ Controls	OR*	95%CI	Cases/ Controls	OR*	95%CI	Cases/ Controls	OR*	95%CI
	LRT p= 0.05						LRT p= 0.04					

*Adjusted for offset terms, age, and gender

§Includes SNPs listed in table and *MTHFR C677T* and *A1298C*; variant allele defined as follows: *MTHFR 677:T; MTHFR 1298:C; SHMT 1420:T; MTRR 66:A*; and *MTR 2756:G*.

£This OR is based on extremely small numbers and thus, is an unstable estimate.

Table 3

Joint effects of individual and combined SNPs and alcohol intake on colon cancer risk by race

	African Americans						Whites					
	Cases/ Controls	OR*	95%CI	Cases/ Controls	OR*	95%CI	Cases/ Controls	OR*	95%CI	Cases/ Controls	OR*	95%CI
	Drinker			Non-Drinker			Drinker			Non-Drinker		
SHMT 1420												
CC	22/18	1.0	Ref	81/129	0.5	0.3, 1.1	52/102	1.0	Ref	95/148	1.3	0.9, 2.0
CT	23/30	0.6	0.3, 1.5	78/98	0.7	0.4, 1.5	57/79	1.4	0.9, 2.3	68/118	1.2	0.7, 1.9
TT	4/12	0.3	0.1, 1.0	23/27	0.8	0.3, 1.8	10/27	0.7	0.3, 1.6	18/42	0.8	0.4, 1.6
CT, TT	27/42	0.5	0.2, 1.2	101/125	0.7	0.4, 1.5	67/106	1.2	0.8, 2.0	86/160	1.1	0.7, 1.7
	ICR= 0.66 (95% CI 0.24, 1.08), p= 0.002						ICR= −0.50 (95% CI −1.28, 0.28), p= 0.21					
	LRT p= 0.04						LRT p= 0.15					
MTRR 66												
GG	4/3	1.0	Ref	19/23	0.7	0.1, 3.7	37/65	1.0	Ref	60/100	1.1	0.7, 1.9
AG	30/32	0.9	0.2, 4.3	64/91	1.7	0.1, 3.3	64/96	1.2	0.7, 2.0	87/151	1.1	0.7, 1.8
AA	15/25	0.6	0.1, 3.0	99/140	1.7	0.1, 3.4	18/47	0.7	0.3, 1.4	34/57	1.1	0.6, 2.0
AG, AA	45/57	0.7	0.2, 3.6	163/231	0.7	0.2, 3.3	82/143	1.0	0.6, 1.7	121/208	1.1	0.7, 1.7
	ICR= 0.26 (95% CI −0.99, 1.51), p= 0.68						ICR= −0.06 (95% CI −0.74, 0.61), p= 0.86					
	LRT p= 0.73						LRT p= 0.85					
MTR 2756												
AA	32/40	1.0	Ref	91/136	0.9	0.5, 1.7	79/137	1.0	Ref	124/195	1.1	0.8, 1.6
AG	16/18	1.3	0.5, 2.9	76/105	1.0	0.6, 1.8	37/66	1.0	0.6, 1.6	50/103	0.9	0.6, 1.4
GG	1/2	0.6	0.1, 7.0	15/13	1.4	0.6, 3.5	3/5	1.1	0.2, 4.7	7/10	1.3	0.5, 3.6
AG, GG	17/20	1.2	0.5, 2.7	91/118	1.1	0.6, 1.8	40/71	1.0	0.6, 1.6	57/113	0.9	0.6, 1.4
	ICR= −0.08 (95% CI −1.09, 0.94), p= 0.88						ICR= −0.19 (95% CI −0.83, 0.46), p= 0.57					
	LRT p= 0.90						LRT p= 0.57					
# Variant Alleles§												
0 or 1	4/5	1.0	Ref	15/28	0.7	0.1, 3.0	8/16	1.0	Ref	27/39	1.4	0.5, 3.7
2 or 3	32/30	1.5	0.4, 6.3	85/132	1.0	0.3, 4.1	68/113	1.2	0.5, 2.9	100/135	1.5	0.6, 3.7
4 or 5	10/22	0.7	0.1, 3.2	76/85	1.3	0.3, 5.3	39/66	1.2	0.5, 3.0	43/121	0.7	0.3, 1.8
6 or more	3/3	1.4	0.2,11.8	6/9	1.1	0.2, 6.0	4/13	0.6	0.3, 2.3	11/13	1.7	0.5, 5.4

	African Americans				Whites			
	Cases/ Controls	OR*	95%CI		Cases/ Controls	OR*	95%CI	
LRT p= 0.23					LRT p= 0.06			

*Adjusted for offset terms, age, and gender

§Includes SNPs listed in table and *MTHFR C677T* and *A1298C*; variant allele defined as follows: *MTHFR 677:T; MTHFR 1298:C; SHMT 1420:T; MTRR 66:A*; and *MTR 2756:G*.